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Researchers Produce Motor Neurons from Embryonic Stem Cells

Beginning with cultured mouse embryonic stem cells, researchers have administered a precise mix of chemical signals to coax the cells to differentiate into functioning motor neurons.

The achievement was made possible by a decade of work in deciphering the signals that trigger differentiation of motor neurons, which are responsible for controlling the movement of muscles. The experiments represent an important step in applying that knowledge to grow functioning neurons from stem cells — undifferentiated cells that have the potential to become many different types of adult cells.

"This is just the starting point for trying to take a rational approach to studying the ability of ES-cell-derived motor neurons to restore function."

Thomas M. Jessell

According to the researchers, the success of the experiments with mouse cells suggests that the same type of approach might be used to grow human motor neurons from stem cells. Such neurons could enable regeneration of nerve tissue lost to disease or trauma.

The experiments by researchers led by Howard Hughes Medical Institute investigator Thomas Jessell at Columbia University were reported in an article that was given immediate early publication status by the journal *Cell* and published online on July 17, 2002. The research was funded in part by Project A.L.S.

For more than 15 years, Jessell and his colleagues have been attempting to untangle the delicate connections of nerve cells in the developing spinal cord. Their studies have shown that the fledgling vertebrate nervous system

is crackling with activity — genes are being turned on and off at a rapid pace, transforming immature cells into a billions-strong network of specialized neural cells. Ultimately, Jessell hopes that his research will provide a more thorough understanding of how the central nervous system (CNS) is constructed — this, he says, may suggest new ways to repair diseased or damaged components of the mature CNS.

According to Jessell, the attempt to generate motor neurons from stem cells relied on many years of research to identify the chemical cues in the developing embryo that coax naïve neuroprogenitor cells to differentiate into spinal cord motor neurons. These chemical signals direct ES cells down a developmental pathway in successive stages —first into neurons and ultimately into ever more specialized spinal cord motor neurons. Two of the key signals, said Jessell, are retinoic acid, which work with the group of Thomas Edlund had shown converts mid-brain neural cells into spinal cord progenitors, and Sonic hedgehog, a protein that converts spinal cord progenitor cells into motor neurons.

Until now, we have been trying to piece together these steps as individual bites, said Jessell. But we hadnt shown that the normal signaling factors could be used conjointly to take a naïve class of progenitors like ES cells, and by sequential exposure to these factors, recapitulate this developmental pathway.

First author Hynek Wichterle, a postdoctoral fellow in Jessells laboratory, began by using retinoic acid and other chemical cues to induce ES cells to differentiate into mid-brain-type neurons and then into spinal cord neurons. The scientists could follow the steps of differentiation by looking for the expression of specific transcription factors that define the identity of cells as spinal cord progenitor cells.

Retinoids will give you spinal cord identity, but that doesnt determine exactly which type of neuron emerges from these spinal cord progenitor cells, said Jessell. And thats where Sonic hedgehog becomes important, because our work and that of others had shown that you need Sonic hedgehog signaling at exactly the right level of signal activation to generate motor neurons.

Thus, when the scientists exposed the cultured spinal cord neurons to appropriate levels of the Sonic hedgehog protein, the cells differentiated to become motor neurons. The dependence of this differentiation on a narrow concentration range of the Sonic hedgehog protein is significant, said Jessell, because in developing embryos the amount of Sonic hedgehog governs what type of neuron will be generated.

In additional experiments, the scientists used ES cells from transgenic mice whose motor neurons were tagged with a fluorescent marker. The fluorescent tagging enabled Jessell and his colleagues to monitor, isolate and purify the

specific motor neurons they had induced — a technique that Jessell believes will be crucial to further attempts to define the signaling pathways involved in neuronal differentiation.

The researchers were also able to address an important question, namely, whether the motor neurons they had developed in culture could actually function in living animals. We needed to demonstrate how well these *in vitro* -generated motor neurons did when they were put into a living embryo, Jessell said. So, Hynek managed the very impressive technical feat of reintroducing these ES-cell-derived motor neurons back into the spinal cords of chick embryos at a stage when normal motor neurons are being generated. The scientists then tested in the chick embryos how well the introduced neurons survived, integrated themselves into the embryonic spinal cord and extended their long cable-like axons toward their normal targets in muscle.

I think our results documented that these ES-cell-derived motor neurons do a pretty good job of mimicking their embryo-derived counterparts in all of those three tests, said Jessell. In general, I was pleasantly surprised by how well neuralized ES cells recapitulate the developmental events that we have come to associate with motor neuron progenitors and motor neurons.

Jessell believes that these successes represent only the beginning of a promising line of research. This is just the starting point for trying to take a rational approach to studying the ability of ES-cell-derived motor neurons to restore function, not just in an embryonic context, but in a more relevant adult context, he said.

Jessell and his colleagues hope to use ES-generated motor neurons in experiments to identify all the genes that govern the pathways of motor neuron differentiation. They are also developing collaborations with neurologists to explore in mouse models whether their motor neurons can regenerate spinal cords that have been damaged by trauma or neurodegenerative diseases, such as amyotrophic lateral sclerosis.

The researchers also plan to explore whether the signaling pathways of motor neuron differentiation mice resemble those in humans. I think one can be cautiously optimistic that such parallels will exist, said Jessell. While many scientists have shown that human ES cells can give rise to neurons, we dont know exactly which type of neurons they are. And, theres a much greater heterogeneity in the properties of human ES cells than in mouse ES cells; so it may be necessary to sift through a number of the available human ES cell lines before arriving at a cell which behaves as its mouse counterpart. But, in principle there is no reason why this type of approach might not be successful with human cells, he said.

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